RESEARCHES IN REGARD TO THE COAGULO REACTION OF THE SYPHILITIC SERUM.

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THE Wassermann test was originally regarded as a specific serum reaction, dependent upon the interaction of antigen and antibody; but the extensive investigations which have since been devoted to this subject resulted in the conviction that the original assumption does not hold good, for it became apparent that lipoid extracts from non-syphilitic organs are as well adapted to demonstrate the reaction as the extracts of syphilitic livers which were employed at first. On the other hand, the extracts from cultures of spirochetes were shown by Noguchi's investigation to be not suitable for the purpose. A second hypothesis set up by Wassermann,[1] according to which in the course of syphilitic infection special antibodies to lipoids appear in the same organism, has also proved to be without foundation.

In addition to the Wassermann test a number of reactions are described which are more or less characteristic of syphilitic serum, and which consist mainly in precipitations which take place in the serum when certain additions are made to it. So far they have not proved very reliable as a means of diagnosing syphilis, since they occur far less regularly than the Wassermann reaction, and often have not the necessary accuracy and clearness.

A year ago Hirschfeld and Klinger[2] mentioned a new test which they christened the coagulo test, because it is built upon the technic of coagulation which they evolved. According to the data of the authors the results of this test are as characteristic as, and not less constant than, those of the Wassermann test. Subsequent experiments carried out by Frenkel and Thiele,[3] and especially by Brandt,[4] who tested a considerable quantity of material with the coagulo reaction, confirmed its utility for the diagnosis of syphilis. Brandt came to the conclusion that in many cases of treated or latent lues the Hirschfeld-Klinger test surpassed the Wassermann test in sensitiveness. From a theoretical point of view also the coagulo test is of interest, since it enables one to recognize the peculiar transformation undergone by the serum in the course of luetic infection on a totally different basis and by means of a widely differing technic.

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[1] München. med. Wchnschr., 1913, p. 1331.

[2] Deutsch. med. Wchnschr., 1914, No. 32. Semaine méd., August 5, 1914.

[3] München med. Wchnschr., 1914, p. 2005.

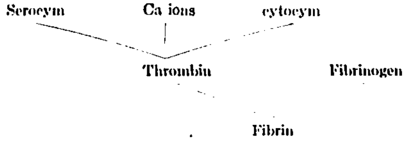
[4] Deutsch. med. Wchnschr., 1915, No. 31.

Hirschfeld and Klinger[5] assume, in explanation of the reaction described by them, that ultramicroscopic precipitates produced by the extract in the serum (particularly in the globulins) enclose the lipoid particles and thereby render them ineffective for coagulation. While the Wassermann test only demonstrates these globulin precipitations indirectly by means of the alterations they induce in the complement, the coagulo test enables one to recognize them at once by the lipoids which participate directly in the process; this circumstance establishes the superiority of the coagulo reaction to the Wassermann reaction.

In this paper I wish to report upon a considerable number of cases which I investigated by means of this new test, comparing, at the same time, my results with those of the Wassermann test. I also experimented whether, by altering the dose of the separate agents, the delicacy of the test could be heightened without its specificity being diminished.

Perhaps I may be permitted to preface my report by a few general facts on the physiology of coagulation which are necessary for the thorough comprehension of what follows:

When blood coagulates two separate processes can be distinguished, namely, the development of thrombin (fibrinferment) and the precipitation by the thrombin of fibrinogen, *i. e.*, the actual coagulation (or formation of fibrin). The thrombin itself consists of two substances, (1) the serocym (thrombogen), which is an albuminous constituent of the blood plasma, and (2) the cytocym (thrombokinase), which belongs to the group of the lipoids, in particular of the lecithins, and which can be extracted from almost any cells by means of alcohol. In the blood one of the chief sources of the cytocym are the blood plates, by the decomposition of which the cytocym requisite for the coagulation of the blood mainly becomes free. If ionized calcium salt is present the serocym and cytocym form thrombin. Once the latter has developed it occasions the precipitation of fibrinogen even in the absence of Ca ions, for instance in oxalate medium. The accompanying scheme will present the processes clearly.



If, therefore, we wish to prove the presence of thrombin in a fluid and to determine its quantity, we only require to mix a definite amount of this fluid with a solution of fibrinogen and to observe when coagulation takes place. This process ensues the more quickly the more thrombin is present, and does not occur at all without thrombin.

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[5] Ztschr. f. Immunit., 1915, xxiv.

In place of the too unstable pure fibrinogen solutions there may with advantage be employed, according to the investigations of Bordet and Delange, a weak solution of oxalate plasma, *i. e.*, plasma obtained by centrifugation of blood mixed with oxalate. Upon the addition of larger quantities of thrombin this oxalate-plasma hardens in from one to two minutes; smaller quantities only cause coagulation after eight to fifteen minutes, whereas mere traces of thrombin only cause incomplete coagulation in the course of several hours. The employment of plasma mixed with oxalate has not only the advantage that this plasma (fibrinogen) solution will keep good a long time, but it also prevents any further formation of thrombin from the moment at which the plasma is added. The reason of this is that the Na oxalate precipitates the lime in the form of insoluble Ca oxalate, and the consequent lack of Ca ions renders any further formation of thrombin impossible. We are thus enabled to determine quantitatively the amount of thrombin present in a fluid at a given moment, also the process of coagulation is divided into two distinct phases, that of the formation of thrombin and that of the proof of thrombin being present, a methodological advance which the physiology of coagulation owes to the researches of Bordet and Delange.

We have seen in the above how thrombin can be determined quantitatively. But for the coagulo test we must be able to measure accurately not only the resulted thrombin, but also one of its constituents, the cytocym. To attain this aim the procedure is as follows: We add to the fluid of which the cytocym content is to be determined a solution of serocym (sheep serum) and a small quantity of $CaCl_2$ solution, both in carefully ascertained amounts. If cytocym is present the formation of thrombin will immediately follow, which will have reached its height in ten to fifteen minutes, and which will be the more vigorous the more cytocym is present. After fifteen minutes we interrupt the formation of thrombin by the addition of oxalate plasma. The thrombin which has formed will cause the coagulation of the latter, which process will be the quicker the more abundantly thrombin had formed. The time required for coagulation is therefore in inverse ratio to the amount of thrombin, *i. e.*, of cytocym.

This method enables one to determine the quantity of coagulo-active lipoids (cytocym) in a fluid not indeed absolutely but yet very exactly in relation to a given cytocym solution. In this quantitative measurement of cytocym we see demonstrated the technical side of the coagulo test described by Hirschfeld and Klinger.

The principle of this test is based upon the fact that most of the alcoholic organic extracts which are employed for the Wassermann test, when emulsionized in the customary manner in saline solution, provide good cytocym, and that the coagulating activity of these extract emulsions is greatly depressed or even wholly arrested by syphilitic sera, whereas normal sera only influence it very slightly. The line of action to be pursued, therefore, consists mainly in digesting for some time a suitable quantity of the serum to be examined with the extract in emulsion, then determining the cytocym content, *i. e.*, the coagulating activity of the extract by comparing it with the activity shown by the same quantity of extract after it has been in contact with normal serum or with saline solution.

TECHNIC. In regard to the preparation of the reagents required for this test the following observations may be of use:

1. *Extract Emulsion.* Only alcohol, not acetone extract, can be employed. Almost every extract may be employed. We generally made use of extracts taken from guinea-pigs' hearts or the human liver. The concentration and effectiveness of the extract should be such that, diluted twenty to forty times and tested in the manner described below, 0.1 c.c. of it causes the oxalate plasma to coagulate in one to three minutes. Of this extract, which can be preserved for an unlimited period if it be kept unpolluted by water (for example, wet pipettes), dilutions should be made of 1 to 40, 1 to 80, 1 to 160, even 1 to 320 in saline solution and 0.1 c.c. of each of these dilutions decanted into glass tubes ready for each serum that has to be examined. Should an extract in a given concentration not be sufficiently active it can be concentrated somewhat more by vaporization of part of the alcohol; it is not advisable to employ for the coagulo reaction extracts the effect of which is too weak. The use of more powerful extract concentrations than those cited above (for instance, 1 to 10) is not permissible on account of the alcohol which affects the formation of thrombin. Considering the extraordinary activity of most organic extracts it is usually easy to procure them in the strength which seems most desirable.

2. *Serocym.* According to Hirschfeld and Klinger the most serviceable serocym solution is sheep or goats' serum, prepared from the oxalated blood of these animals in the following way: From the compressed jugular vein blood is drawn off in a jet, not drop by drop, by means of a fairly thick cannula and conducted into a glass retort containing 1 per cent. Na oxalate solution. As this solution is not isotonic for all animals we recommend adding to it one-twentieth volume of a 10 per cent. saline solution. The blood is allowed to flow into the retort until it reaches a certain mark made beforehand on the latter, and which shows that the blood now contains 1 per mil. oxalate, *i. e.*, the oxalate solution has been diluted ten times over by the blood. During the process of taking the blood everything must be avoided which might favor the formation of thrombin. The blood as it flows into the retort should be shaken slightly so that it mixes immediately with the oxalate. It is important that the con-

densation of water vapor from the warm blood on the glass walls of the retort be prevented, as otherwise partial hemolysis easily occurs, the plasma becomes red and is consequently useless. This condensation can be sufficiently arrested by heating the oxalate solution and the retort to about 40° C. and thoroughly moistening the inner walls of the retort with the blood as quickly as possible. Blood obtained in this manner does not coagulate and must next be centrifugated in centrifugal glasses which have been previously rinsed in saline solution (this again to avoid condensation); after ten to fifteen minutes the plasma, now free from blood, must be pipetted off into fresh glasses and vigorously centrifugated for at least a half hour in order to remove the blood plates. The resulting plasma should be absolutely clear, and if colored at all, of a yellow shade, by no means reddish. From it we procure the serum serocym by means of recalcification to produce coagulation. We add one-tenth volume of a 1 per cent. $CaCl_2$ solution, which causes the whole to become much clouded, and leave it to stand for a quarter hour, preferably in a temperature of 37° C. The plasma coagulates during this time and must now be taken hold of by one side with long forceps (about 35 cm. long) and pressed dry by a wringing movement. If the plasma has not coagulated at all, or only partially after the lapse of ten to fifteen minutes, the addition of a few more drops of $CaCl_2$ solution usually suffices to bring about rapid coagulation. Plasmas which will not coagulate at all, or only do so very slowly in spite of a sufficient addition of Ca (and this often happens in the case of plasma which have been kept for some time), often do not produce usable serocym at all. After being pressed dry the serocym must be left to stand for some time. It then frequently coagulates again, in which case it must be pressed dry again. Freshly dried serocym still contains a little thrombin, so should not be used for testing purposes until after the lapse of one to two hours. For the tests it must be diluted with five times its volume of physiological saline solution and employed in the proportion of 0.5 c.c. per tube. Good serocym should contain neither cytocym nor thrombin. For every test it must be controlled in this direction. The dose to be employed must be put into the tube with the $CaCl_2$ without cytocym, and, mixed with oxalate plasma, should not coagulate, at least not until the following day. The procuring of good serocym is the only preparation in connection with the coagulation test, which is attended by some slight difficulty. But after a little practice it becomes quite easy to procure serum of the required quality. Certainly it may occur that serocym which has been obtained with all due observance of rules and regulations is nevertheless ineffective, *i. e.*, only generates in conjunction with the cytocym a small amount of thrombin. The primal cause of this phenomenon is not yet fully clear. Experiments which I made in this direction showed that the serocym content of the blood not only varies from animal to animal, but is even subject to variation in one and the same animal; nor could I discover that this occurrence has any connection with any physiological process, such as digestion, etc. Often blood which had been taken from a sheep in the afternoon was very rich in serocym, while a specimen taken in the morning had only provided very inferior serocym. Many sheep have habitually such inferior serocym that their blood cannot be employed for illustrating the reaction. Weak serocym can be considerably improved by leaving it to stand for some hours in ten times the volume of distilled water *instead of immediately adding saline solution to it as directed above.* Before the solution (it will be cloudy on account of the globulin precipitate) can be used, 10 per cent. saline solution must be poured into it until it contains 0.8 per cent. of salt, after which it may be employed in doses of 1.

Serocym taken from other animals in use in laboratories is less well adapted for our purpose; rabbits, indeed, provide serviceable serocym, but it would be difficult to procure it in the required quantity. Guinea-pigs, oxen, and human beings have not a sufficient amount of serocym.

3. *$CaCl_2$ Solution.* This is a physiological saline solution to which are added 5 per cent. of a 1 per cent. $CaCl_2$ solution.

4. *Oxalate Plasma.* For this the oxalate plasma designed to provide the serocym can be used, but as it must be available in considerable quantities it is advisable to obtain the blood of some large animal in oxalate direct from the incision (1 per cent. oxalate). It must be centrifugalized twice over as soon as possible to remove all the blood plates. Frequently when plasma obtained in this manner has stood for one to two days, coagula appear in it, caused by small amounts of thrombin reaching the blood in spite of all precautions. These coagula, which may not be too copious, must be strained off by filtering (through cotton-wool) until the plasma should be quite clear; if this is the case it can be preserved fit for use for one or two weeks if kept in a refrigerator. When in use it must be diluted as follows: 1 part plasma, 5 parts physiological saline solution, ½ part 1 per cent. Na oxalate solution. In the following this mixture will merely be designated oxalate plasma.

5. *Preparation of the Serum.* Some of the patient's blood must be collected in a centrifuge tube and treated in the same manner as for Wassermann test. The serum must be well centrifugated and should not be tinged red to any appreciable degree by dissolved corpuscles (see below). As the serum resulting from the spontaneous coagulation of blood always contains a certain amount of cytocym, which would disturb the coagulo reaction, the sera intended for the test must be thoroughly inactivated; preferably for an hour in a temperature of 56° C., whereby the cytocym of the serum will be destroyed.

Carefully handled, sera are usually free of cytocym after heating for half an hour; unskilfully obtained specimens of blood, on the other hand (such as have been violently shaken on the transit for instance), produce sera that are very rich in cytocym, and consequently require to be heated for an hour up to 58 or 60°C.

DEMONSTRATION OF THE REACTION. The coagulo test can be carried out most conveniently by using a stand in which can be placed a considerable number of tubes in rows, each row containing four or five tubes. Each serum must be tested with three or four different doses of cytocym. A corresponding number of dilutions of the organic extract (see above) must be prepared, for instance, in the proportion of 1 to 40, 1 to 80, 1 to 160, and 1 to 320. Of each of these 0.1 c.c. must be decanted into a separate tube and 0.1 c.c. eventually 0.2 c.c. (see below) of the inactive serum added to it.

For the control the same dose of the serum alone (without the extract) must be left to stand in 0.1 saline solution; similarly the doses of extract employed must be placed in the control tubes alone, *i. e.*, without the addition of serum. The tubes must next be well shaken and then left to stand half to one hour at room temperature. During this time the reaction between serum and cytocym will take place. At this stage of the procedure we add to the contents of each tube, including the controls, 0.5 c.c. of the serocym dilution and 1 c.c. $CaCl_2$—NaCl solution; mix them thoroughly and again leave the tubes to stand for fifteen minutes (formation of thrombin). At the end of this time 1 c.c. oxalate plasma must be added everywhere, mixed at once with the other contents and the time of coagulation noted. If the reagents have been skilfully placed in the tubes and the serocym is good the controls should coagulate after one or three to five minutes; negative sera postpone the time of coagulation for some minutes while positive sera definitely inhibit coagulation. The control containing serum alone should remain liquid, as here no cytocym was added; if one of these tests also coagulates (autocoagulation) it is a sign that the serum itself still contained some cytocym (probably through being unskilfully procured). Such sera are useless for demonstrating the coagulo reaction. This autocoagulation has nothing in common with the auto-inhibition which occurs sometimes when the Wassermann test is applied, for if a second specimen of the same patient's blood is taken and more carefully handled the serum can always be used.

Below I insert two protocols of experiments which illustrate the times of coagulation characteristic for positive and negative sera. From them it is apparent that not every dose of cytocym affords serviceable figures, for, proportionate to the strength of the serocym, etc., sometimes the larger, sometimes the smaller, amounts of cytocym furnish the determining factors for a diagnosis.

PROTOCOL I.

Extract dilution.	Extract without serum.	Control sera.		1	2	3	4	5	6	7	8	9	10
		+	−										
1: 40	1½'	5'	2½'	3½'	3½'	2½'	1½	5'	2'	2	30'	2½'	2'
1: 120	2'	30'	4'	22'	fl.	3'	3'	fl.	2'	3'	fl.	30'	3½
1: 240	2'	fl.	5'	fl.	fl.	15'	4½	fl.	3'	5'	fl.	fl.	4½
Serum without extract	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	5
Result	....	....	....	+	+	±	−	+	−	−	+	+	A
W. R.	....	....	....	±	+	?	−	+	−	−	+	+	+
Clinical diagnosis:	....	....	....	Iritis.	Iritis.	Orchitis.	Syphilis.	......	Lues latens.	Lues III.	Lues III.	Progressive paralysis.	Lues III.

PROTOCOL II.

Extract dilution.	Extract without serum.	Control sera.		1	2	3	4	5	6	7	8	9	10
		+	−										
1: 40	1'	2'	1½'	1½'	2½'	2	3'	1½'	2'	3'	1½'	2'	1½
1: 80	1'	5'	2'	2'	5'	4	22'	2'	2'	5'	2'	2'	2'
1: 160	1½'	15'	3'	3'	18'	15	fl.	3'	3'	14'	4'	3'	4'
Serum without extract	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.
Result	....	....	....	−	+	+	+	−	−	+	−	−	−
W. R.	....	....	....	−	+	+	+	−	−	+	−	−	−
Clinical diagnosis:	....	....	....	Lues latens.	Lues I.	Lues latens, treated.	Tabes dorsalis.	Neurasthenia.	Tabes dorsalis, incipient.	Progressive paralysis.	Lues latens, treated.	Paranoia.	Wet-nurse.

W. R. = Wassermann reaction. fl. = fluid. + = positive. ± = weak positive. A = autocoagulation.

PROTOCOL III.—A. PRELIMINARY ARRANGEMENT.

Extract dilution.	Extract without serum.	Positive serum, 0.1 c.c.	Weak positive serum, 0.1 c.c.	Negative serum, 0.1 c.c.
1: 40	2′	15′	7′	4′
1: 120	4′	30′	20′	6′
1: 240	6′	45′	30′	15′
Serum without extract	fl.	fl.	fl.	fl.

PROTOCOL III.—B. PRINCIPAL REACTION.

Extract dilution.	Extract without serum.	Serum doses.																					
		1		2		3		4		5		6		7		8		9		10		11	
		0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
1: 40	2′	2′	4	3′	5	1h	fl.	4′	6′	2′	5′	2′	3′	3′	3′	3	3	2	5	2	5	2	5
1: 120	4′	1h	fl.	16′	fl.	fl.	fl.	5′	30′	12′	20′	4′	5′	16′	18′	10	45	6	18	4	6	5	8
1: 240	6′	fl.	fl.	fl.	fl.	fl.	fl.	25	fl.	30′	fl.	7′	10′	18′	30′	15	fl.	fl.	fl.	6	12	fl.	fl.
Serum without extract	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.	fl.
Results	. . .	+		±		+		—		—		—		±		—		—		—		—	
W. R. o	. . .	—		±		+		—		—		—		—		—		—		—		—	
W. R. n	. . .	—		+		+		—		—		—		±		—		—		—		—	
Clinical diagnosis:	. .	Lues II, treated.		Tabes dorsalis, treated.		Lues ?		Phthisis, pulmonary.		Tumor at tonsilla.		Lues healed.		Lues.		Polyneuritis.		Iridocyclitis.		Keratitis.		Iridocyclitis.	

W. R. o = means old methods; W. R. n = new method (see below).

In Protocol I the positive sera (third row of tubes) have completely destroyed the cytocym of the smallest dose of extract ($\frac{1}{240}$). A slightly positive serum, No. 3, responding uncertainly to the Wassermann test, coagulates in fifteen minutes, while the time required by the negative sera is only three to five minutes (in conjunction with extract alone four minutes). With a double quantity of extract the difference is still clearly discernible with the exception of Nos. 3 and 10, when they can no longer be sufficiently distinguished. The first row, on the other hand, containing one-fortieth extract, does not furnish figures serviceable for differentiating between positive and negative.

In the second protocol the serocym was still stronger, the gradation of the doses of extract not being carried so far. The positive sera consequently did not cause absolute inhibition, but only a protraction of the time equaling ten to fifteen minutes. In the second row the differences are just intimated, in the first scarcely recognizable.

The third protocol illustrates the furthest extreme in the opposite direction. Here the serocym was so weak that in the third row even many negative sera (like Nos. 4 and 5) caused considerable delay in the time of coagulation. Most of the tubes in this row remained liquid. Here diagnosis must be based on the second row, where the time limit for positive sera is fifteen minutes. In the first row only the positive sera (positive in response to the older style of Wassermann test) show definite results.

We shall refer again to the figures contained in this table, which were obtained with 0.2 c.c. serum.

To this protocol is prefixed a preliminary experiment which was carried out in the morning to test the reliability of the serocym prior to the demonstration of the principal reaction (of which protocol III B only reproduces a small part). Since it sometimes happens, as already mentioned, that the serocym only causes very slight formation of thrombin, it is advisable to determine each time its quality. The serocym, diluted 1 to 4, must be placed in the tubes in a dose of 0.5 c.c., together with doses of extract, arranged in a falling scale, eventually also with a positive and a negative serum; according to the thus ascertained strength of the serocym the doses of extract can be more or less graduated in the main experiment. To guard against failure when experimenting on a large scale it is well to employ four rather than only three different doses of extract (1 to 40, 80, 160, 320), even though this precaution necessarily rather increases the number of tubes.

The inserted protocols seem to make it clear that the difference between positive and negative sera is only quantitative. In the case of the coagulo test (just the same as when the Wassermann test or any other test based on serum reaction is applied) only positive sera cause the appearance of definitely typical signs (such as the plasma remaining fluid instead of coagulating), while the numerous slightly positive sera, or such as show a mere trace of luetic infection, can only be classified by the help of collateral comparisons and special reflexion. It is necessary, therefore, always to experiment simultaneously with a positive and a negative serum, and especially to employ some slightly positive sera as controls, after which a careful comparison of all the results must take place. It is also desirable always to apply the coagulo test to a considerable number of sera at once, as it is then far easier, especially with the additional help that may be afforded sometimes by the anamnesis of particular patients, to decide upon the border cases, whether they are to be ranked as negative or just positive.

Altogether I subject to the coagulo test 500 sera which had been sent to the institute for examination by the Wassermann test and compared the two sets of results. The *résumé* in Protocol IV shows how this material was classified in accordance with the clinical aspect of the respective case, and to which extent the results of the two methods of testing coincided.

Agreement between the two reactions under comparison was attained in 92.75 per cent. of the cases if we do not exclude cases where merely the strength of the reactions differed (+ and =). In regard to the remaining 7.25 per cent. the coagulo test cannot be said to have failed throughout, since a number of cases belong here in regard to which the anamnesis supports the testimony of the coagulo test rather than of the Wassermann test and thus establishes the superiority of the former to the latter (positive, generally ±) according to coagulo test, negative according to Wassermann test in 13 cases of treated lues and suspected lues, in 5 cases of latent lues, and in 12 cases in which the anamnesis indicated other symptoms of syphilis). On the other hand there were only 8 cases which resulted negatively in the coagulo test, while the clinical aspect and the Wassermann test pointed to syphilis. In the case of these few sera (as in that of the autoprecipitations), in all probability, not the coagulo test as such was at fault, but awkwardness in the method of procuring and despatching the specimens of blood, an important factor to which we shall again refer.

If we only include those cases in which the clinical aspect pointed to probable lues (269 cases) the coagulo test was positive in 173 cases (64.7 per cent.) and the Wassermann test only in 154 cases (57.2 per cent.); this signifies a superiority of the former on the latter of 7.5 per cent.

In explanation of the table under the title of "various affections" were examined, among others, one case of psoriasis and one of lichen, both negative. One case which clinically was described as sporotrichosis was positive both in the coagulo and in the Wassermann test, and therefore doubtless of syphilitic origin. Among the group of wet-nurses were 2 cases which reacted slightly positively in the coagulo test, though they responded negatively to the Wassermann test and had no mention of lues in their record. The serum of women before and after childbirth also showed in some cases a certain tendency to inhibit the activity of cytocym more than other normal sera, but always only in a limited degree. Similar observations have been made in patients suffering from tumor, for instance, in a case of tumor on the kidney, which is included in the table. Whether in these two conditions positive coagulo reaction really occurs frequently can only be proved by further careful investigation.

PROTOCOL IV.

Clinical diagnosis.	Reaction.	Positive.	Weak positive.	Negative.	Number of cases.	Conformable.
Lues I	W.	6	3	8	17	16
	C.	5	5	7		
Lues II	W.	18	5	20	43	42
	C.	17	5	21		
Lues III	W.	11	2	5	18	18
	C.	9	4	5		
Lues	W.	17	0	23	40	39
	C.	16	2	22		
Lues latent	W.	8	8	31	50	47
	C.	8	11	31		
Lues treated	W.	7	2	38	47	43
	C.	8	3	36		
Lues hereditary	W.	2	1	5	8	7
	C.	2	2	4		
Lues cerebral	W.	0	0	4	4	3
	C.	1	0	3		
Tabes dorsalis	W.	6	1	7	14	13
	C.	6	2	6		
Progressive paralysis	W.	9	0	4	13	12
	C.	9	1	3		
Lues, questionable	W.	21	10	77	108	104
	C.	20	15	73		
Iritis, iridocyclitis	W.	4	0	13	17	14
	C.	4	3	10		
Keratitis parenchymatosa	W.	4	0	3	7	7
	C.	3	1	3		
Non-syphilitic eye diseases	W.	0	0	9	9	9
	C.	0	0	9		
Indefinite syphilitic diseases of central nerve system	W.	0	0	12	12	10
	C.	1	1	10		
Clinical, not syphilitic infectious diseases	W.	0	0	9	9	9
	C.	0	0	9		
Aortic insufficiency	W.	0	1	4	5	5
	C.	1	0	4		
Tumor	W.	0	0	5	5	4
	C.	0	1	4		
Chronic nephritis	W.	0	0	3	3	3
	C.	0	0	3		
Pulmonary tuberculosis and lues	W.	2	1	2	5	4
	C.	2	2	1		
Tuberculosis	W.	0	0	7	7	6
	C.	1	0	6		
Lupus	W.	0	0	1	1	1
	C.	0	0	1		
Addison's disease	W.	0	0	1	1	1
	C.	0	0	1		
Anemia	W.	0	0	3	3	3
	C.	0	0	3		
Faults of hearing	W.	0	0	6	6	5
	C.	0	1	5		
Various affections	W.	1	0	16	17	17
	C.	1	0	16		
Diseases without diagnosis	W.	4	2	14	20	20
	C.	3	3	14		
Diabetes mellitis	W.	0	0	4	4	4
	C.	0	0	4		
Wet-nurse	W.	2	0	13	15	13
	C.	2	2	11		
Healthy	W.	0	0	3	3	3
	C.	0	0	3		

W = Wassermann reaction. C = coagulo reaction.

The sensitiveness and accuracy of the coagulo test are dependent in the first instance from the amount of cytocym employed. By graduating the dilutions of extract more elaborately than Hirschfeld and Klinger originally suggested (the second and third row of tubes now contained instead of ½ and ¼, ⅓ and ⅙ of the dose of extract employed in the first row) I succeeded in intensifying the sensitiveness of the coagulo test still further. This is obvious at once if we compare it with the Wassermann test results. Brandt, who worked with less diluted cytocym, attained agreement between his results and those of the Wassermann test in 94 per cent. of the cases under examination.

He had compared his results with those of the Wassermann test as it was carried out at that time in the institute[6] according to the scheme in general use (0.1 serum, 0.05 complement, three different extracts in single and double, not autoinhibiting doses). Meanwhile a change of technic was introduced in the institute which, judging from our experiences up to the present time, increased the sensitiveness of the Wassermann test by about 10 per cent.

Starting from the well-known fact that hemolysis takes place more quickly in the tubes containing negative sera plus extract than in the controls containing extract alone, Frenkel (in Heiden) suggested going a step further: employing the extract in an autoinhibiting dose and adding to it relatively large amounts of serum (up to 0.5 c.c.). Negative sera prevent autoinhibition while positive sera increase it.

I compared my results with those afforded by this elaborated complement-binding test which, as mentioned above, in cases of syphilis furnishes positive results about 10 per cent. more frequently than the old method. As, even after this innovation, I attained agreement between the coagulo test and the Wassermann test quite as often as Brandt (94 per cent.), this would seem to prove that the further the graduating of the extract doses is carried the more sensitive is the test.

In a number of experiments I tried putting larger doses of the serum under examination into the tubes as well as the customary quantity (0.1), and investigated whether by this means more definite results might be reached in slightly positive or questionable cases. The greater the quantity of serum added to a constant dilution of cytocym the less effective the latter becomes for the coagulo test. 0.1 c.c. of normal serum compared with the control (saline solution) almost always shows delay of coagulation for some minutes. If 0.2 to 0.5 c.c. serum are employed the delay is far more pronounced. No increase in the sensitiveness of the reaction on luetic sera was observed in general as a result of employing these larger amounts of serum (see for instance Protocol III, for which all the sera had been tested in double as well as the ordinary quantities). If the doses of serum amount to 0.3 c.c. or more, even normal sera not infrequently show apparently positive results; with doses of 0.2 c.c. the reaction was in some cases more sharply defined than with 0.1 c.c. In other experiments this was not the case, the interpretation of the results was, on the contrary, sometimes rendered more difficult by unspecific inhibition being observed in normal sera. It seems best, consequently, to apply the test with 0.1 c.c. of serum. In experiments with only a few sera I would suggest filling a second row of tubes with the 0.2 c.c. dose of each serum.

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[6] He also made his investigations in the institute of Hygiene at Zurich.

PROTOCOL V.

Clinical diagnosis.	Lues, infection one year ago.		Lues II.		Lues I-II.		Ulcer of anus.		Acute otitis.		Ulcer on penis.		No diagnosis.		Extract without serum.
W. R.	+		+		+		−		−		−		−		
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Extract dilution:															
1: 40	2'	2'	3'	2'	3	3	1'	1'	1'	1'	1'	1'	1'	1'	1'
1: 80	3'	3'	3'	3'	14	3	1'	1'	1½'	1'	3'	1'	1'	1'	1'
1: 160	4'	4'	12'	3½'	15	3½	1½'	3'	3'	2'	5'	3'	1½'	1'	1'
1: 320	fl.	5'	15'	5	1.	5	1½'	4'	10'	4'	25'	10'	4'	2'	2'
Serum without extract . .	fl.	5'	1.	fl.	1.	fl.	1.	fl.	1.	fl.	fl.	fl.	fl.	5	fl.
Results	+	A	+	−	+	−	−	−	−	−	±	−	−	A	

Series a = without the addition of blood.
Series b = with the addition of blood.
A = autocoagulation.
W. R. = Wassermann reaction.

In the course of our experiments we were struck by the fact that the sera which reacted slightly positively in the Wassermann test and negatively in the coagulo test were often colored a deepish red by hemoglobin. This led us to investigate whether the admixture of small definite quantities of dissolved blood could affect the sera in such a way as to diminish their inhibition in regard to cytocym. This is actually the case, as the following protocol shows. For the practical illustration of this phenomenon it is necessary to add the blood to the serum before the latter is inactivated and then to heat the two together. The reaction is not affected if blood, heated separately, is added to the serum after its inactivation. Also, the quantity of blood must not be too great, as then, on account of the lipoids content of the blood corpuscles, the serum becomes too rich in cytocym, which in its turn leads to autoprecipitation (Nos. 1 and 7 in the protocol).

Both positive and negative sera, as classified by the Wassermann test, should be inactivated after the customary manner, some with and some without the addition of 1 to 3 drops of washed human blood corpuscles (a 5 per cent. suspension in saline solution), and then subjected to the coagulo test.

The result shows that in every case in which blood was added inhibition was lowered, a condition which would require us to declare serum heated together with blood as no longer positive, but negative.

I wish to lay particular stress on this source of possible errors (which has been overlooked by earlier investigators), because sera more or less tinged with red in consequence of the blood having been carelessly drawn, or the specimen violently shaken on the transit are not all uncommon and might occasionally account for the fact of a slightly positive reaction in a serum not being registered by the coagulo test. If the latter were applied to irreprovable material only I doubt if it would ever fail.

SUMMARY. In summarizing the results of my investigations I may safely assert that the coagulo reaction is highly characteristic in the case of syphilitic serum, especially when the elaborated method described above is applied, and the sources of possible mistakes which we have also mentioned carefully avoided. In many cases this test is distinctly superior to the Wassermann reaction. After some practice one easily gains sufficient mastery over the technic on which it is based to be able to examine even a large number of sera without difficulty. It is hardly probable that the coagulo reaction will finally supercede the time-honored Wassermann reaction; but it does seem calculated both to furnish scientific research with valuable data in regard to the transformation of serum as wrought by syphilis, and also to facilitate the diagnosis of doubtful cases of syphilis, possibly also of certain apparently similar conditions.

THE NERVOUS SYMPTOMS OF POLYCYTHEMIA VERA.[1]

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It seems worth while to emphasize the nervous symptoms presented by patients with polycythemia vera, not because the case reports and various studies of this condition fail to make mention of their frequent occurrence, but because a failure to keep them in mind very often has led to diagnostic mistakes and in some instances to cerebral operations, with the idea that the symptoms were the result of brain tumor.

If one has in mind the very striking appearance of some patients of this group with their marked cyanosis or, probably better, reddish cyanosis it would seem as if this disease would be recognized promptly; but, alas, how often do we fail to see what is just in front

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[1] Read at the meeting of the Association of American Physicians, May, 1917.